EFFICACY OF BOTANCAL AND MINERAL OILS ON WILLAMETTE MITE
(ACARI: TETRANYCHIDAE)

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Elizabeth Ruby Begonia Church
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COMMITTEE MEMBERSHIP

TITLE: Efficacy of Botanical and Mineral Oils on Willamette Mite
(Acari: Tetranychidae)

AUTHOR: Elizabeth Ruby Begonia Church

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COMMITTEE CHAIR: Michael J. Costello, PhD.

COMMITTEE MEMBER: Jeff C. Wong, PhD.

COMMITTEE MEMBER: Margaret Rice, PhD

Abstract

Efficacy of Botanical and Mineral Oils on Willamette Mite (Acari: Tetranychidae)

By

Elizabeth R.B. Church

Willamette mite (WM), *Eotetranychus willamettei*, is a major pest throughout most winegrape regions in coastal California and Oregon. These mites puncture leaf tissue with their chelicerae and cause loss of photosynthetically active area. Chemical control treatments on grape include nearly ten registered synthetic miticides, plus soaps and oils. Oils can be petroleum based (mineral oil) or botanical (from seeds of various plants). There has been a lot of interest of late in the use of botanical oils other than soybean, including those derived from the seeds of plants including spearmint, rosemary and clove. This project tested for differences in the efficacy of a mineral vs. a botanical oil. The botanical oil was a blend of rosemary and peppermint oil (Ecotrol®) and was tested against a petroleum based oil (Omni Oil®), then compared to a commonly used synthetic miticide (Nexter®, common chemical name pyridaben). The field experiment (San Juan Vineyards in Shandon, CA) tested effects on adult mite and egg populations, with five treatments: Omni Oil®, Ecotrol®, Nexter®, Ecotrol® + Nexter®, and water as a control. Laboratory experiments tested effects on adult females and eggs. Treatments were Omni Oil®, Ecotrol® (1.0%), Ecotrol® (0.5%) and water as a control. Field data showed that Omni Oil® (at 1.5%) was the only effective treatment, Ecotrol® (at 0.5% or 1.0%) did not differ from the control. Omni Oil® was the most effective treatment

against adult mites in the laboratory studies, followed by Ecotrol® at 1.0%, with Ecotrol® at 0.5 % not very effective. Egg mortality was high with Omni Oil® 1.5% or Ecotrol® 1.0% but low with Ecotrol® 0.5%.

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Introduction

Mites

The Willamette spider mite (WM), *Eotetranychus willamettei* (MacGregor) (Acarina: Tetranychidae), is considered a major pest of grapes throughout much of coastal California, the west side of the San Joaquin Valley, the Sierra Foothills and eastern Tulare and Kern counties, as well as Oregon. Feeding by these mites results in a loss of chlorophyll, causing leaf yellowing and affecting the photosynthetic ability of the plant if mite populations are high.

Conventional management for Willamette spider mite (WM) relies heavily on chemical miticides. Several synthetic miticides are used on grapes in California, including propargite, fenbutatin-oxide, pyridaben, fenpyroximate, and abamectin. In 2006, 21,791 pounds of propargite were applied on wine grapes in California, 533 pounds of fenbutatin-oxide, 7,835 pounds of pyridaben, 2,479 pounds of fenproximate, and 316 pounds of abamectin (CDPR). Synthetic pyrethroids (such as permethrin) have been documented to cause outbreaks of Pacific spider mite and Willamette mite due to toxicity and repellency toward phytoseiid mites which are predators of WM and Pacific spider mite (Gerson and Cohen, 1989 and Hoy et al., 1979), and the factor of hormoligosis.

Spider mite control is particularly problematic because the mites can have up to 10 generations per year, which can accelerate the development of a miticide-resistant population. The Pacific spider mite, *Tetranychus pacificus* (MacGregor), is a more damaging grape pest compared to *E. willamettei*, and has a distribution that is limited to

the San Joaquin Valley south of Lodi, and the warmer regions of the North Coast and Central Coast. It is a more severe pest than the WM due to its rapid rate of populations increase and the vine necrosis it can cause when present in high numbers.

Botanical essential oils

Pesticide resistance is an important issue in agriculture both for mites and insects, in all settings (stored product, greenhouse, and field). It is hoped that botanical oils can offer alternative options to synthetic pesticides and contribute to pesticide resistance. However, it has been found that some resistant strains of insects are also resistant to botanical oils, possible due to cross-resistance. An example of this is the chlorpyrifos-methyl resistant strain of *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle which showed at least 2 times the amount of resistance to *Eucalyptus globules* (Labill) oil as a non-resistant strain in a fumigation study (Lee et al., 2000).

Aromatic plants that produce volatile organic compounds (VOCs) such as terpenes and phenolics that are found in botanical oils, have long been used for their medicinal, perfumery, and food qualities (Regnault-Roger, 1997). Volatile compounds provide the characteristic odor to certain plant tissues. Due to the potency of these compounds, they are generally sequestered or compartmentalized in the plant to prevent autotoxicity (Schoonhoven et al., 2005). These VOCs are also part of the phytochemistry referred to as secondary phytochemicals, and are used in inter-and intraspecific communication (i.e. allelochemicals and pheromones, respectively). Allelochemicals are biological chemicals that are used by plants to attract pollinators or natural enemies,

or repel or immobilize other herbivores. VOCs function by attracting pollinators and defending against or repelling herbivores, fungi and microorganisms, and they also help to prevent plants from water loss by preventing excessive evaporation (Renault-Roger, 1997).

Essential oils are what give plants (such as lemon) their characteristic odor (Cseke and Kaufman, 1999); these are often terpenes and terpenoids, which are common lipids in aromatic plants. Examples are 1,8-cineole, a monoterpenoid, which is shared by rosemary (*Rosmarinus officinalis* L.), marjoram (*Origanum marjorana* L.), and some other members of the Lamiaceae (mint family) (Regnault-Roger, 1997), and limonene, a monoterpene, which is present in significant (>30%) amounts in celery and dill, members of the Apiaceae (carrot family) (Regnault-Roger, 1997). Botanical essential oils are generally obtained by steam distillations of plant material, usually leaves and seeds, but can also be isolated through water distillation or controlled instantaneous decomposition (Boutekedjiret et al., 2004).

Botanical oils and insects

The mode of action of essential oils, such as rosemary oil, on arthropods, is largely unknown, due to the complexity of the chemical constituents. There are ten major constituents in rosemary essential oil, with the terpene 1,8-cineole as the most abundant compound (31.5%) (see Table 1) (Miresmailli et al., 2006).

Constituent	% v/v
Camphene	8.0
1,8-Cineole	31.5
β - Pinene	6.8
Camphor	20.0
<i>p</i> - Cymene	0.9
Borneol	1.2
D- Limonene	3.7
α -Terpineol	1.1
Bornyl acetate	2.2
α -Pinene	17.5
Other compounds	7.2

Table 1. Major constituents of *Rosmarinus officinalis* (L.) essential oil and their relative proportions in the pure oil (Miresmailli et al., 2006).

These oils may operate through more than one mode of action due to the diversity of terpenes and terpenoids (or other secondary compounds) in each plant extraction (Chaisson et al., 2004). Essential oils are generally active on a broad spectrum of arthropods, but the toxicity of individual compounds may be specific to a narrower range or the interaction between an arthropod and the chemical constituent (Isman, 2000). The more that is known about the capabilities of botanical essential oils against arthropod pests the better, because the oils should be used in conjunction with other chemical or biological control tactics (Miresmailli et al 2006).

Some studies have attempted to determine the modes of action of botanical essential oils and their constituents on arthropods. Essential oils of eugenol, α -terpineol,

and cinnamic alcohol were tested on the American cockroach (*Periplaneta americana* L.), the German cockroach (*Blattella germanica* L.) and the carpenter ant (*Camponotus* DeGeer) to determine a mechanism of action (Enan 2001). Results showed that the compounds in essential oils were neuro-insecticides, were species dependent in regard to efficacy, had a synergistic efficacy when used in combination, and that the octopaminergic system is what mediates the insecticidal activity. Results also showed that a blend of eugenol, α -terpineol, and cinnamic alcohol was more effective on *P. americana*, *B. germanica*, and *C. pennsylvanicus* than either α -terpineol or cinnamic alcohol (Enan 2001). Octopamine acts as a neurohormone, a neuromodulator and a neurotransmitter in invertebrates and modulates nearly every physiological process in the central nervous system. The octopaminergic system is thought to be a good target for pesticides because it appears to be much more important in invertebrates than in vertebrates (Roeder, 1999).

Essential oils such as marjoram, rosemary, and peppermint (*Mentha piperita* L.) are effective against some arthropod pests (such as the two-spotted spider mite) in direct contact and fumigant trials (Jang et al., 2005, Miresmailli et al., 2006, and Choi et al., 2004). Another means of determining efficacy of essential oil toxicity is through laboratory studies by determining LD₅₀/LC₅₀, such as with catnip (*Nepeta cataria* L.) and cedarleaf (*Thuja occidentalis* L.) toxicity on the Egyptian cotton leafworm (*Spodoptera littoralis* Boisduval) with LC₅₀ ≤ 10.0 ml/m³ (Pavela, 2005).

Research has been conducted using the specific constituents of essential oils for pest control in the form of fumigation, for example, with major stored-grain coleopteran insects such as the red flour beetle (*Tribolium castaneum* Herbst) and the lesser grain

borer (*Rhyzopertha dominica* F.) (Lee et al., 2004). Essential oils of *Eucalyptus nicholii* (Maiden and Blakely) and *Melaleuca fulgens* were found to have high amounts of the constituent 1,8-cineole (82.19% and 77.50% respectively) and were found to be some of the most toxic against *T. castaneum* and *R. dominica*. The fumigation toxicity at LC₅₀ was measured at using µl/L of air. For *T. castaneum*, the essential oil of *E. nicholii* had a LC₅₀ of 13.7 µl/L of air, while *M. fulgens* had a LC₅₀ of 14.1 µl/L of air. For *R. dominica*, *E. nicholii* had a LC₅₀ of 9.5 µl/L of air, while *M. fulgens* had a LC₅₀ of 7.8 µl/L of air. In this experiment, 1,8-cineole was tested as an individual constituent against other essential oils, including the *E. nicholii* and *M. fulgens*. 1,8-cineole was found to have an LC₅₀ of 15.3 µl/L and 9.5 µl/L of air against *T. castaneum* and *R. dominica* respectively (Lee et al., 2004).

Repellent and oviposition deterring effects on the common greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) caused by the oil of *Pongamia pinnata* L. have also been studied (Pavela and Herda, 2007). Results showed that the pongam oil was a successful repellent (based on whether whiteflies settled on chrysanthemum leaves or not) because untreated plants were preferred. It was also an effective oviposition deterrent with between 80% and 100% deterrence, regardless of oil concentration (0.5 to 2 %) (Pavela and Herda, 2007). With both repellence and oviposition deterrence, the effects decreased over time due to evaporation and limited persistence of botanical oils on plants.

Essential oils and leaves of the Lamiaceae plant family have been used as oviposition deterrents on stored products. Specifically, the leaves of *Minthostachys* spp. have been found to deter the oviposition of *Phthorimaea operculella* (Zeller) (potato

tuber moth) by 80% when spread on potato tubers in storage (Guerra et al., 2007).

The monoterpenoids thymol, citronellal, and eugenol, as well as the complex essential oil of rosemary, have been studied on the larval form of *Agriotes obscurus* L. (Dusky wireworm) (Waliwitiya et al., 2005). In this study, both contact and fumigant toxicities were measured after 24 hours. The contact/topical bioassay found that thymol, citronella and eugenol were the most toxic with LD₅₀ values of 196, 405, and 517 µg/larvae respectively, while rosemary oil only achieved only 24% mortality at 1600 µg/larvae. In the fumigation bioassay, all compounds (citronellal, rosemary oil, thymol and eugenol) showed toxicity with LC₅₀ of 6.4, 15.9, 17.1, and 20.9 µg/cm³.

Orange oil extract (containing ~92% d-limonene) has been tested against *Coptotermes formosanus* (Shiraki), the Formosan subterranean termite for mortality in contact and vapor experiments (Raina et al., 2007). In the laboratory studies there was 96% mortality over 5 days when the orange oil at 5 ppm was dispensed from above, allowing gravity to disperse the oil. In this experiment, a model wall was built in order to mimic structure pest infestations. Vapor exposure within this model wall caused very little mortality, although it did cause worker termites to eat less. The contact study involved orange oil extract treated sand that the termites crawled through. In this section of the study, termites did not tunnel the length of the tube and all died before crossing one third of the length. A drawback is that in three weeks the traces of orange oil extract were negligible, showing fast degradation.

Horticultural mineral oils have shown a lack of toxicity from topical application and residue on parasitoids such as *Colpoclypeus florus* (Walker) (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Nagarkatti) (Hymenoptera: Trichogrammatidae) (Brunner et al., 2001).

Botanical oils and mites

Essential oils from aromatic plants have been recently researched as potential miticides (Aslan et al., 2003). Much research has been conducted involving the two-spotted spidermite (Koch) (*Tetranychus urticae*), a significant greenhouse and field pest worldwide. Essential oil vapors from summer savory (*Satureja hortensis* L.) (Lamiaceae) has shown to be effective in controlling motile stages of *T. urticae* in a greenhouse setting (Aslan et al., 2003). Essential oil vapors from hyssop (*Micromeria fruticosa* L.), catmint (*Nepeta racemosa* L.) and Greek oregano (*Origanum vulgare* L.) have been tested for insecticidal and acaricidal efficacy against *T. urticae* and *Bemisia tabaci* Genn (Calmasur et al., 2006). *Tetranychus urticae* adults and/or nymphs mortality rates were the highest (96.7, 95 and 95%) at the highest treatment rate (2 µl/l) for vapor exposure time of 120 hours for *M. fruticosa*, *N. racemosa*, and *O. vulgare* respectively (Calmasur et al., 2006). For *B. tabaci*, mortality was 100% at 120 hours at 2 µl/l for all essential oils. Epazote (*Chenopodium ambrosioides* L.) essential oils have also been found to be effective against *T. urticae* (Chaisson et al., 2004). An emulsifiable concentrate UDA-245 with 25% *C. ambrosioides* essential oil extract (at 0.5%), had a 97.5% mortality on adult *T. urticae* (Chaisson et al., 2004).

A variety of botanical essential oils was tested by Choi et al (2004) on *T. urticae*, using a filter paper diffusion bioassay. This study found that caraway seed, geranium,

lemon eucalyptus, lemongrass, oregano, pennyroyal, peppermint, sage and spearmint caused 100% mortality at a dose of $19 \times 10^{-3} \mu\text{l} / \text{mL}$ air. At $7.1 \times 10^{-3} \mu\text{l} / \text{mL}$ air, lemon eucalyptus essential oil still caused > 85% mortality in *T. urticae* (Choi et al., 2004).

In a different study, rosemary oil was tested on *T. urticae* by painting the leaf disk resulting in an LC_{50} of $10.0 \mu / \text{liter}$ for adult females on beans and $13.0 \mu / \text{liter}$ on tomatoes (Miresmailli et al., 2006). 100% mortality of *T. urticae* was achieved with rosemary oil at $20 \mu / \text{liter}$ on beans and $40 \mu / \text{liter}$ on tomatoes after 24 hours. When constituents of the rosemary essential oil were tested individually, 1,8 cineole and α – pinene were found to be the most toxic to adult female *T. urticae*, although the greatest mortality was achieved with a full mixture of the rosemary constituents (Miresmailli et al., 2006).

Clove bud (*Eugenia caryophyllata*) oil was tested as a fumigant against house dust mites (*Dermatophagoides* spp), and as a direct contact toxin giving 100 % mortality at a dose of $12.7 \mu\text{g} / \text{cm}^2$ 24 hours after treatment (Kim et al., 2003). Cypress (*Chamaecyparis obtuse* Kiso-Hanoki) oil was tested as a fumigant against (*Dermatophagoides pteronyssinus* and *Dermatophagoides farina* Hughes) (Jang et al., 2005). Sixteen volatile compounds were extracted from the cypress leaves and β -thujaplicin was found to be the most toxic (based on LC_{50}) compound against both mite species (Jang et al., 2005).

Mortality of the stored food mite, *Tyrophagus putrescentiae* Schrank (Acari: Acaridae) was tested against seven botanical essential oil monoterpenoid compounds (pulegone, eucalyptol, linalool, fenchone, menthone, α -terpinene, and γ -terpinene) (Sanchez-Ramos and Castanera, 2001). A mortality of >90% was achieved with these

monoterpenes at a concentration of 66.7 µl/l under vapor conditions. The most effective of those terpenes were pulegone and menthone with LC₅₀ of 3.7 and 4.7 µl/l respectively. The eggs of *T. putrescentiae* were not significantly affected by these compounds (Sanchez-Ramos and Castanera, 2001).

There is understandable concern regarding how botanical oil based miticides might also affect predatory mites, especially the commercially available and widely used *Phytoseilus persimilis* Athias-Henriot. Choi et al (2004) found that peppermint oil at 4.7×10^{-3} µl/ml air in particular was highly toxic to *P. persimilis* with > 90% mortality. Pure rosemary oil has also been found to have an effect on *P. persimilis* with an LC₅₀ of 16.62 ml/l (Miresmailli and Isman, 2006).

On the other hand, in one study, Ecotrol® at the recommended rate (0.5%) had no mortality effect on *Phytoseilus persimilis* Athias-Henriot) in direct spray laboratory experiments (Miresmailli et al., 2006). This suggests that the use of botanical oil based pesticides may be conducive to maintaining natural enemy populations.

The intention of this study was to determine the efficacy of mineral and botanical based oils in the control of WM in both adult and egg stages on wine grapes. There has been little research conducted on Willamette mites in regard to alternative miticides, although there is need for better control strategies due to increasing regulation and the lack of miticides available for organic growers. Field research on WM is also limited, therefore field and laboratory studies were conducted.

Materials and Methods

Field study

The study site was a block of *Vitis vinifera* (cv. Chardonnay) at the San Juan Vineyards in Shandon, San Luis Obispo County, California. During the experiment (August-September 2006), the average high and low temperatures at the site were 33.49° and 11.67° C (92.3° and 53° F) respectively. Spacing at the site was 3.63 m (12 feet) between rows and 1.52 m (5 feet) between vines within the row.

A randomized complete block design was used (see Figure 1) with four replications of five treatments. The rows on either side of the experimental row, as well as more than 20 vines on each end, were not sprayed with a miticide during the season this experiment took place. Each plot consisted of 4 in-row vines, with the inner two vines used for sampling. Myclobutanil (Rally®) and azoxystrobin (Abound®) were applied to the block at standard rates for control of powdery mildew (*Erisiphe necator*). No other insecticides or miticides were used on this block for the duration of the season.

The experimental five treatments consisted of petroleum oil (Omni Oil®), an essential oil blend (Ecotrol®), pyridaben (Nexter® - a commonly used miticide), combination of Nexter® and Ecotrol® (label rate are presented, Table 2), and water as a control. The essential oils in Ecotrol® are from rosemary (10%), peppermint (2%) and a small percentage of wintergreen (*Gaultheria fragrantissima*). The adjuvant Natural Wet® was included with the Ecotrol® and Omni Oil® treatments. Natural Wet® is a biodegradable antistress wetting agent consisting of a formulation of Yucca plant (*Yucca schidigera* L.) saponins (10%).

Figure 1. Plot map of experimental design at research site.

A Maruyama Mist Duster model MD 155DX (Maruyama Mfg. Co., Tokyo, Japan) was used to apply all of the treatments. The first treatment date for all treatments was August 16, 2006 (treatment summary is in Table 2). After the second day of sampling, the Ecotrol® plots were sprayed again with a double concentration, and the control plots were sprayed again with water (sprayed August 24, 2006). All miticide concentrations are volume/volume (v/v), except pyridaben which is weight/volume (w/v).

Leaf samples were taken approximately every three days, starting with a pre-

treatment count on August 11 and continuing on through September 5. From the inner two vines of each plot, ten leaves were sampled from the lower third of the canes with five leaves taken from each side of the row. Leaves were placed in labeled paper bags, grouped in a plastic bag and placed in an ice chest, then transported to the laboratory on the California Polytechnic State University campus.

	Application Dates	
	1 st Application 8/16/2006	2 nd Application 8/24/2006
Treatment		
Control (water)	934 L/ha	934L/ha
Ecotrol®	0.5%(v/v) (4.67 kg/ha) (4 pints/ac)	1.0% (v/v) (6.35 L/ha) (8 pints/ac)
Nexter®	0.519 L/ha (7 oz/ac)	
Nexter® + Ecotrol®	Nexter®: 0.519 kg/ha Ecotrol®: 0.5% (v/v)	
Omni Oil®	1.5% (v/v) 14.03 L/ha (1.5 gal/ac)	
Adjuvant (Natural Wet®)	*Used with Ecotrol® and Omni Oil® treatments 1 L/800L (16 fl oz/100gal)	

Table 2. Treatment summary, rates and application dates

Leaf samples were processed using a mite brushing machine (Leedom Enterprises,

Miwok Village, CA) and WM motiles and eggs were counted under a dissecting microscope, counting from the inner three rings of the grid (see Figure 2), which amounted to approximately 20% of the disk. Mites and eggs per leaf were estimated using the formula determined by Macmillan (2005): $y = (4.538x + 2.28)/10$, where x represented the raw disk count.

Figure 2. Mite counting grid.

Laboratory study

Laboratory research was conducted at the California Polytechnic State University, San Luis Obispo entomology laboratory. Miticide trials were conducted on WM adult females and eggs. All miticide concentrations were v/v.

For the adult female study, there were four trials with all four field treatments (control, Ecotrol® 0.5%, Ecotrol® 1.0% and Omni Oil® @1.5%) (Natural Wet® adjuvant, was included for the Ecotrol® and Omni Oil® treatments as it was in the field study). Each treatment was replicated five times, and conducted four times (four separate trials). Each replication consisted of 10 adult female mites per leaf disk (1.9 cm

diameter/ 0.75 inches diameter) on a tray, and therefore 50 mites per treatment per trial.

The egg study had five trials with the first three consisting of only three treatments (control, Ecotrol® @ 0.5%, and Omni Oil® @ 1.5%) and the last two with all four treatments (control, Ecotrol® @ 0.5%, Ecotrol® @ 1.0% and Omni Oil® @ 1.5%). Treatments are summarized in Table 3.

Mites for the laboratory study were taken from area vineyards (San Juan and Arciero Vineyards in Paso Robles). Watch glasses (small 5 cm diameter glass dishes with curved edges) with cotton batting cut to fit the bottom were used for this experiment. For the egg study, one leaf disk was placed on a moistened cotton round in a watch glass for each replication, and adult females were placed on the leaf disks and allowed to lay approximately ten eggs, and the adults were then removed. For the adult trials, 10 adult female mites were removed from a leaf with a fine hair brush and placed on leaf disk. Before the miticides were applied, each disk was checked to ensure that 10 live female mites or 10 intact eggs were present.

Treatments were applied using a Crown Spra-Tool (Crown, Woodstock, IL), an aerosol canister with attached container for the treatment solution. Treatments were sprayed one tray at a time under a fume hood, using five dishes per tray. Post application trays were placed in a growth chambers (Percival Scientific, Perry, IA), with temperature set at 25° C (77° F) with a 14-hour photoperiod in order to simulate field conditions and to not stimulate diapause in the adult females. Treatments were not mixed within each chamber, ie. a separate chamber was designated for each treatment. Experiments were then checked on a twenty-four hour period for one week.

For the adult trials, mortality was noted and summarized as mean percent mortality per disk. Adult females were considered dead when they were dry and discolored. If dead, they were removed from disks. For the egg trials, hatch was noted and recorded as mean percent hatch. Once hatched, juveniles were removed from the disks.

Experiment Type	Trial	Date	Treatments (control, Omni Oil, Ecotrol 0.5%, Ecotrol 1.0%)
Adult female	1	9-12	All 4
Adult female	2	9-20	All 4
Adult female	3	9-28	All 4
Adult female	4	10-11	All 4
Egg	1	8-19	Only 3 (only 0.5% concentration of Ecotrol®)
Egg	2	8-27	Only 3
Egg	3	9-1	Only 3
Egg	4	9-10	4
Egg	5	9-23	4

Table 3. Treatment summary for laboratory study.

Statistical analysis

Field counts of mites and eggs were analyzed by analysis of variance (ANOVA) separating means by orthogonal contrasts (SAS Institute Inc, 2002). The data were log 10 transformed to stabilize the variance.

Laboratory adult mortality data were analyzed using repeated measures analysis of variance (ANOVA), and laboratory egg hatch data were analyzed using ANOVA (SAS Institute Inc, 2002).

Results

Field Studies

Density of motile stages

On 17 August, one day after the first treatment (DAT1), ANOVA showed a significant difference (95% confidence) in mite density (Table 4). There was a significant difference between Omni Oil® and the control, and between Nexter® and the control, but not between the control and Ecotrol® (0.5%), or the Nexter®/Ecotrol® (0.5%) combination. The mite density with Omni Oil® was 79.1% lower compared to the control, (Figure 3).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	1.08	0.27	5.84	0.0076
Block	3	0.1104	0.0368	0.80	0.5195
Error	12		0.0462		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	0.1276	0.1276	2.76	0.1225
Omni Oil® vs control	1	0.7639	0.7639	16.52	0.0016
Nexter® vs control	1	0.2671	0.2671	5.78	0.0333
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.0189	0.0189	0.41	0.5343

Table 4. ANOVA from the field study, with dependent variable $\log_{10}$ (mites per leaf), on 17 August, one day after the first treatment. (SS stands for Sum of Squares)

On 21 August, five DAT1, ANOVA showed a significant difference in mite density (Table 5). There was a significant difference between Omni Oil® and the control, and between Nexter® and the control, but not between the control and Ecotrol® (0.5%) or the

Nexter®/Ecotrol® (0.5%) combination. The mite density with Omni Oil® was 83% lower and Nexter® was 71% lower compared to the control (Figure 3).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	2.391	0.5978	10.44	0.0007
Block	3	0.5035	0.1678	2.93	0.0768
Error	12		0.0573		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	0.1598	0.1598	2.79	0.1207
Omni Oil® vs control	1	1.822	1.822	31.82	0.0001
Nexter® vs control	1	0.9026	0.9026	15.76	0.0019
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.1514	0.1514	2.64	0.1299

Table 5. ANOVA from the field study, with dependent variable $\log_{10}$ (mites per leaf), 21 August, five days after the first treatment.

On 25 August, one day after second treatment (DAT2) (the control and Ecotrol® @ 1.0%), ANOVA showed a significant difference in mite density (Table 6). There was a significant difference between Omni Oil® and the control, and between Nexter® and the control, but not between the control and Ecotrol® (1.0%) or the Nexter®/Ecotrol® (0.5%) combination. The mite density with Omni Oil® was 95.4% lower and Nexter® was 94.4% lower compared to the control (Figure 3).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	6.102	1.525	14.43	0.0002
Block	3	0.2723	0.0908	0.86	0.4889
Error	12		0.1057		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(1.0%) vs control	1	0.155	0.155	1.47	0.2493
Omni Oil® vs control	1	2.746	2.746	25.97	0.0003
Nexter® vs control	1	2.766	2.766	26.17	0.0003
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.0812	0.0812	0.77	0.3979

Table 6. ANOVA from the field study, with dependent variable $\log_{10}$ (mites per leaf), 25 August, one day after the second treatment.

On 28 August, four DAT2, ANOVA showed a significant difference in mite density (Table 6). There was a significant difference between Omni Oil® and the control, and between the control and Ecotrol® (1.0%), but not between Nexter®, or the Nexter®/Ecotrol® (0.5%) combination. The mite density with Omni Oil® was 84.5% lower, and Ecotrol® (1.0%) was 73.5% lower compared to the control (Figure 3).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	3.682	0.9204	9.47	0.0011
Block	3	0.7772	0.2591	2.67	0.0951
Error	12		0.0972		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(1.0%) vs control	1	0.6806	0.6806	7.01	0.0213
Omni Oil® vs control	1	2.100	2.100	21.62	0.0006
Nexter® vs control	1	0.3897	0.3897	4.01	0.0683
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.027	0.027	0.28	0.6076

Table 7. ANOVA from the field study, with dependent variable $\log_{10}$ (mites per leaf), 28 August, four days after the second treatment.

On 5 September, eleven DAT2, ANOVA showed a significant difference in mite density (Table 8). There was a significant difference between Omni Oil® and the control, and between Nexter® and the control, but not between the control and Ecotrol® (1.0%) or the Nexter®/Ecotrol® (0.5%) combination. The mite density with Omni Oil® was 84.9% lower and Nexter® was 74.6% lower compared to the control (Figure 3).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	2.225	0.5563	14.80	0.0001
Block	3	0.4956	0.1652	4.40	0.0264
Error	12		0.0376		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(1.0%) vs control	1	0.0714	0.0714	1.90	0.1932
Omni Oil® vs control	1	0.7901	0.7901	21.02	0.0006
Nexter® vs control	1	1.337	1.337	35.56	<.0001
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.0533	0.0533	1.42	0.2566

Table 8. ANOVA from the field study, with dependent variable $\log_{10}$ (mites per leaf), 5 September, eleven days after the second treatment.

Figure 3 shows mites per leaf ($\pm$ standard error of the mean) by sampling date in the field study. This graph shows the change in mite density over the period of the field study for each of the five treatments. Overall this graph shows that there was a decrease in mite densities after the initial application of all treatments, but that the Ecotrol® (0.5%) and the control did not sustain a decrease in mite density after Aug 17. A second application of Ecotrol® at an increased rate (1.0%) and the control was applied on Aug 24 to attempt to decrease mite densities in those treatments. This graph shows that the second application did initially decrease the mite densities, but overall the Ecotrol® (0.5%) was not significantly different from the control.

Figure 3. Mites per leaf ($\pm$ standard error of the mean) by sampling date, field study. The concentration of Ecotrol® was 0.5% at first treatment application, and 1.0% at second treatment application.

Analysis based on percent difference from pre-count

Motile stages

Compared to the pre-application count, mite density in the Omni Oil® treatment was 77.4%, 66.3%, 93.3%, 91.9%, and 94.6% lower on 1, 5, 9, 12 and 17 DAT1, respectively (Table 9). The Nexter® treatment had a mite density decrease of 62.5%, 76.7%, 96.7%, 97% and 96.3% on 1, 5, 9, 12 and 17 DAT1, respectively (Table 9). The Nexter®/Ecotrol® (0.5%) combination saw mite density decrease by 56.2%, 82.1%, 94%, 98.1% on 1, 5, 9, 12 and 17 DAT1, respectively (Table 9).

Mite density in the Ecotrol® (0.5%) treatment was 37.49% lower than the pre-count on 1 DAT1, but 13.23% higher on 5 DAT1 (Table 9). After the second application of Ecotrol® @ 1.0%, mite density decreased by 29%, 79% and 49% on 1, 4 and 9 DAT2, respectively (Table 10). The control (water) showed a 37.1% decrease 1 DAT1, but an increase of 15.7% on 5 DAT1 (Table 9). After the second application, mite density in the control decreased 26.8%, 59.6% and 82%, on 1, 4 and 9 DAT2, respectively (Table 10).

Treatment	17 Aug	21 Aug	25 Aug	28 Aug	5 Sept
Control (water)	-37.13	+15.7	-	-	-
Ecotrol® (0.5%)	-37.49	+13.23	-	-	-
Nexter®	-62.53	-76.7	-96.73	-97.0	-96.33
Nexter® + Ecotrol® (0.5%)	-56.2	-82.05	-93.98	-98.13	-95.57
Omni Oil®	-77.44	-66.32	-93.26	-87.54	-94.61

Table 9. Percent difference of mean mites per leaf from pre-count (14 Aug), after first miticide application (16 Aug.) on successive sampling dates for each treatment. There are no results for the control and Ecotrol (0.5%) on 25 Aug., 28 Aug. and 5 Sept., because the second miticide application these treatments occurred on 24 Aug (Table 10).

Treatment	25 Aug	28 Aug	5 Sept
Control (water)	-26.82	-59.59	-82.00
Ecotrol® (1.0%)	-29.00	-79.00	-49.00

Table 10. Percent difference of mean mites per leaf from the 21 Aug. count, after the second miticide application (24 Aug.) on successive sampling dates for control and Ecotrol® treatments. On 24 Aug. Ecotrol® was applied at 1.0%.

Eggs

There was no significant difference among treatments in egg density on 1 DAT1 nor 5 DAT1 (17 Aug. and 21 Aug., respectively) (Tables 11 and 12, respectively; Figure 4).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	0.3965	0.0991	2.09	0.1459
Block	3	0.1504	0.0502	1.05	0.4042
Error	12		0.0475		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	0.0022	0.0022	0.05	0.8348
Omni Oil® vs control	1	0.1231	0.1231	2.59	0.1335
Nexter® vs control	1	0.0927	0.0927	1.95	0.1880
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.1699	0.1699	3.57	0.0831

Table 11. ANOVA from the field study, with dependent variable $\log_{10}$ (eggs per leaf), on 17 August, one DAT1.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	0.9078	0.2269	2.90	0.0682
Block	3	0.1976	0.0659	0.84	0.4967
Error	12		0.0782		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	0.3047	0.3047	3.89	0.0719
Omni Oil® vs control	1	0.6209	0.6209	7.94	0.0155
Nexter® vs control	1	0.0557	0.0557	0.71	0.4153
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	0.0562	0.0562	0.72	0.4132

Table 12. ANOVA from the field study, with dependent variable $\log_{10}$ (eggs per leaf), on 21 Aug., five DAT1.

On 25 August, one DAT2, ANOVA showed a significant difference in egg density (Table 13). There was a significant difference between Ecotrol® (1.0%) and the control, Nexter® and the control, and Nexter® and the Nexter®/Ecotrol® (0.5%) combination and the control, but not between the control and Omni Oil®. Egg density with Ecotrol® (1.0%) was 33.84% lower, Nexter® was 80.04% lower compared to the control. The Nexter®/Ecotrol® (0.5%) combination was 27.27% lower than Nexter® alone (Fig. 4).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	3.838	0.9594	8.78	0.0015
Block	3	0.6164	0.2055	1.88	0.1869
Error	12		0.1093		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(1.0%) vs control	1	1.596	1.596	14.60	0.0024
Omni Oil® vs control	1	0.1992	0.1992	1.82	0.2020
Nexter® vs control	1	0.6676	0.6676	6.11	0.0294
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	2.138	2.138	19.55	0.0008

Table 13. ANOVA from the field study, with dependent variable log 10 (eggs per leaf), on 25 August, one day after the second treatment.

On 28 August, four DAT2, ANOVA showed a significant difference in egg density (Table 14). There was a significant difference between Nexter® and the control, and Nexter® and the Nexter®/Ecotrol® (0.5%) combination, but not between the control and Omni Oil® or Ecotrol® (1.0%). Egg density with Nexter® was 80.77% lower compared to the control. The Nexter®/Ecotrol® (0.5%) combination was 1.5x lower than Nexter® alone (Fig. 4).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	3.086	0.7714	5.40	0.0101
Block	3	0.6437	0.2146	1.50	0.2644
Error	12		0.143		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(1.0%) vs control	1	0.2502	0.2502	1.75	0.2105
Omni Oil® vs control	1	0.1298	0.1298	0.91	0.3595
Nexter® vs control	1	0.7404	0.7404	5.18	0.0420
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	2.299	2.299	16.08	0.0017

Table 14. ANOVA from the field study, with dependent variable log 10 (eggs per leaf), on 28 August, four days after the second treatment.

On 5 September, eleven DAT2, ANOVA showed a significant difference in egg density (Table 15). There was a significant difference between Ecotrol® (1.0%) and the control, and Nexter® and the control, and the Nexter®/Ecotrol® (0.5%) combination and Nexter®, but not between the control and Omni Oil®. Egg density with Ecotrol® (1.0%) was 35.7% higher, and Nexter® was 94.89% lower compared to the control. Nexter® was 60% higher than the Nexter®/Ecotrol® (0.5%) combination (Fig. 4).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	4	3.126	0.7815	10.82	0.0006
Block	3	0.7717	0.2572	3.56	0.0475
Error	12		0.0723		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(1.0%) vs control	1	0.9632	0.9632	13.33	0.0033
Omni Oil® vs control	1	0.1122	0.1122	1.55	0.2364
Nexter® vs control	1	1.733	1.733	23.98	0.0004
Nexter® vs Ecotrol®(0.5%) + Nexter®	1	1.243	1.243	17.21	0.0014

Table 15. ANOVA from the field study, with dependent variable log 10 (eggs per leaf), on 5 September, eleven days after the second treatment.

Figure 4 shows eggs per leaf ($\pm$ standard error of the mean) by sampling date in the field study. This graph shows the change in egg density over the period of the field study for each of the five treatments. Overall this graph shows that there was a decrease in egg densities after the initial application of all treatments, but that the Ecotrol® (0.5%) and the control did not sustain a decrease in egg density after Aug 17. A second application of Ecotrol® at an increased rate (1.0%) and the control was applied on Aug 24 to attempt to decrease egg densities in those treatments. In the egg field study, it was found that there was a general trend of egg density decline as shown in this graph. This graph shows that the second application did initially decrease the egg densities, but overall the Ecotrol® (0.5%) was not significantly different from the control.

Figure 4. Eggs per leaf ($\pm$ standard error of the mean) by sampling date, field study. The concentration of Ecotrol® was 0.5% at first treatment application, and 1.0% at second treatment application.

Compared to the pre-application count, egg density in the Omni Oil® treatment was 12.9%, 69.37%, 96.14%, 91.77%, and 61.44% lower on 1, 5, 7, 9, 12, and 17 DAT1, respectively (Table 16). The Nexter® treatment had an egg density decrease of 9.46%, 38.45%, 91.86%, 95.35%, and 99.22% lower on 1, 5, 7, 9, 12, and 17 DAT1, respectively (Table 16). The Nexter®/Ecotrol® (0.5%) combination saw an initial increase in egg density by 45.26% (one DAT1). But thereafter there was a decrease 49.09%, 95.71%, 98.91%, and 99.45% on 5, 9, 12, and 17 DAT1, respectively (Table 16).

Egg density in the Ecotrol® (0.5%) treatment was 5.6% higher than the pre-count on one DAT1, but 24.9% lower at 5 DAT1 (Table 16). After the second application of Ecotrol® @ 1.0%, egg density decreased 38.5%, 61.85%, and 53.00% on 1, 4 and 9 DAT2, respectively (Table 17). The control (water) showed a decrease of 30.96% and 22.83% on 1 and 5 DAT1, respectively (Table 16). After the second application, egg density decreased by 41.03%, 65.02%, and 78.02% on 1, 4, and 9 DAT2, respectively (Table 17).

Treatment	17 Aug	21 Aug	25 Aug	28 Aug	5 Sept
Control (water)	-30.96	-22.83	-	-	-
Ecotrol® (0.5%)	+5.60	-24.9	-	-	-
Ecotrol® (0.5%) + Nexter®	+45.26	-49.09	-95.71	-98.91	-99.45
Nexter®	-9.46	-38.45	-91.86	-95.35	-99.22
Omni Oil®	-12.90	-69.37	-96.14	-91.77	-61.44

Table 16. Percent difference of mean eggs per leaf from pre-count (14 Aug.) on successive sampling dates for each treatment. Miticide application was on 16 Aug. On 25 Aug., 28 Aug. and 5 Sept. there are no results for the control and Ecotrol (0.5%) because re-application occurred for these treatments on 24 Aug.

Treatment	25 Aug	28 Aug	5 Sept
Ecotrol® (1.0%)	-38.2	-61.85	-53.00
Control (water)	-41.3	-65.02	-78.02

Table 17. Percent difference of mean eggs per leaf from the last count before the second application (24 Aug.) on successive sampling dates for the control and Ecotrol® treatments. On 24 Aug., Ecotrol® was applied at 1.0%.

Lab Studies

Motile stages

One day after treatment (DAT), ANOVA showed a significant difference in motile mite mortality (Table 18). There was a significant difference between the control and Ecotrol® (0.5%), between Omni Oil® and Ecotrol® (1.0%), and between Ecotrol® (0.5%) and Ecotrol® (1.0%). Mortality was higher with Ecotrol® (0.5%) compared to the control by 16.5 percentage points, Omni Oil® compared to Ecotrol® (1.0%) by 26 percentage points, and Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 34 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	70533.75	23511.25	41.79	<0.0001
Error	73		562.62		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs Control	1	2722.5	2722.5	4.84	0.0310
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	11560	11560	20.55	<0.0001
Omni Oil® vs Ecotrol®(1.0%)	1	6760	6760	12.02	0.0009

Table 18. ANOVA from the lab study, with dependent variable percent mite mortality, 1 DAT.

Two DAT, ANOVA showed a significant difference in adult mite mortality (Table 19).

There was a significant difference between the control and Ecotrol® (0.5%), between Omni Oil® and Ecotrol® (1.0%), and between Ecotrol® (0.5%) and Ecotrol® (1.0%).

Mortality was higher with Ecotrol® (0.5%) compared to the control by 15 percentage points, Omni Oil® compared to Ecotrol® (1.0%) by 19 percentage points, and Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 30.5 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	50985	16995	35.98	<0.0001
Error	73		472.4		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	2250	2250	4.76	0.0323
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	9302.5	9302.5	19.69	<0.0001
Omni Oil® vs Ecotrol®(1.0%)	1	3610	3610	7.64	0.0072

Table 19. ANOVA from the lab study, with dependent variable percent mite mortality, 2DAT

Three DAT, ANOVA showed a significant difference in adult mite mortality (Table 20).

There was a significant difference between the control and Ecotrol® (0.5%), between Omni Oil® and Ecotrol® (1.0%), and between Ecotrol® (0.5%) and Ecotrol® (1.0%).

Mortality was higher with Ecotrol® (0.5%) compared to the control by 14 percentage points, Omni Oil® compared to Ecotrol® (1.0%) by 18.5 percentage points, and Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 22.5 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	35413.75	11804.58	23.21	<.0001
Error	73		508.7		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	1960	1960	3.85	0.0535
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	5062.5	5062.5	9.95	0.0023
Omni Oil® vs Ecotrol®(1.0%)	1	3422.5	3422.5	6.73	0.0115

Table 20. ANOVA from the lab study, with dependent variable percent mite mortality, 3DAT

Four DAT, ANOVA showed a significant difference in adult mite mortality (Table 21). There was a significant difference between Ecotrol® (0.5%) and Ecotrol® (1.0%), but not between the control and Ecotrol® (0.5%) or between Omni Oil® and Ecotrol® (1.0%). Mortality was higher with Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 22.5 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	27153.75	9051.3	19.24	<.0001
Error	73		470.4		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	1440	1440	3.06	0.0844
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	5062.5	5062.5	10.76	0.0016
Omni Oil® vs Ecotrol®(1.0%)	1	1562.5	1562.5	3.32	0.0725

Table 21. ANOVA from the lab study, with dependent variable percent mite mortality, 4DAT.

Five DAT, ANOVA showed a significant difference in adult mite mortality (Table 22).

There was a significant difference between Ecotrol® (0.5%) and Ecotrol® (1.0%), but not between the control and Ecotrol® (0.5%) or between Omni Oil® and Ecotrol® (1.0%). Mortality was higher with Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 26 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	26140	8713.3	21.85	<.0001
Error	73		398.8		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	1000	1000	2.51	0.1176
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	6760	6760	16.95	<.0001
Omni Oil® vs Ecotrol®(1.0%)	1	640	640	1.60	0.2092

Table 22. ANOVA from the lab study, with dependent variable percent mite mortality, 5DAT

Six DAT, ANOVA showed a significant difference in adult mite mortality (Table 23).

There was a significant difference between Ecotrol® (0.5%) and Ecotrol® (1.0%) and between the control and Ecotrol® (0.5%), but not between Omni Oil® and Ecotrol® (1.0%). Ecotrol® (0.5%) compared to the control by 11.5 percentage points Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 14.5 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	13890	4630	13.93	<.0001
Error	73		332.3		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	1322.5	1322.5	3.98	0.0498
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	2250	2250	6.77	0.0112
Omni Oil® vs Ecotrol®(1.0%)	1	562.5	562.5	1.69	0.1974

Table 23. ANOVA from the lab study, with dependent variable percent mite mortality, 6DAT

Seven DAT, ANOVA showed a significant difference in adult mite mortality (Table 24).

There was a significant difference between Ecotrol® (0.5%) and Ecotrol® (1.0%), but not between the control and Ecotrol® (0.5%) or between Omni Oil® and Ecotrol® (1.0%). Mortality was higher with Ecotrol® (1.0%) compared to Ecotrol® (0.5%) by 17.5 percentage points.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	14985	4995	12.88	<.0001
Error	73		387.7		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	902.5	902.5	2.33	0.1314
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	3062.5	3062.5	7.90	0.0063
Omni Oil® vs Ecotrol®(1.0%)	1	562.5	562.5	1.45	0.2323

Table 24. ANOVA from the lab study, with dependent variable percent mite mortality, 7DAT.

Overall, ANOVA using repeated measures showed there to be a significant difference between treatments (Table 25). There was a significant difference between Ecotrol®(0.5%) and the control, between Ecotrol®(0.5%) and Ecotrol®(1.0%), and between Omni Oil® and Ecotrol® (1.0%).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	220941.96	73647.32	30.77	<.0001
Trial	3	40870.54	13623.52	5.69	0.0015
Error	73	174740.18	2393.7		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	11188.93	11188.93	4.67	0.0339
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	40320	40320	16.84	0.0001
Omni Oil® vs Ecotrol®(1.0%)	1	14001.43	14001.43	5.85	0.0181

Table 25. Repeated measures ANOVA from the lab study, with between subject effects

Treatment	Mean	Standard Error
Omni Oil®	95.86	1.147
Ecotrol 1.0%®	81.71	2.04
Ecotrol 0.5%®	58.79	2.52
Control (water)	45.29	2.48

Table 26. Percent mortality overall on adult female mites.

Figure 5 shows percent mortality of adult females over time in the laboratory study. This graph shows adult female mortality over the course of seven days for each of the four treatments. This graph shows that the Omni Oil® treatment had a large initial mortality effect on the mites, while Ecotrol® (1.0%) had a moderate initial mortality effect but concluded the study with a high mortality. Both Ecotrol® (0.5%) and the

control had a low initial mortality effect and concluded with a moderate morality.

Figure 5. Laboratory study, percent mortality of adult females over time.

Egg Study

Seven days after treatment, ANOVA showed a significant difference in the treatments (Table 27). There was a significant difference between Ecotrol®(0.5%) and Ecotrol®(1.0%), but not between Ecotrol® (0.5%) and the control, or between Omni Oil® and Ecotrol® (1.0%). Percent hatch was 31.25 percentage points lower with Ecotrol® (1.0%) than with Ecotrol® (0.5%) (Figure 6).

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Treatment	3	68096.12	22698.71	31.66	<.0001
Trial	3	8693.31	2897.77	4.04	0.0076
Error	378		716.9		
Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Ecotrol®(0.5%) vs control	1	1390.07	1390.07	1.94	0.1646
Ecotrol®(0.5%) vs. Ecotrol®(1.0%)	1	16844.11	16844.11	23.50	<.0001
Omni Oil® vs Ecotrol®(1.0%)	1	639.98	639.98	0.89	0.3453

Table 27. ANOVA from lab egg study.

Figure 6 shows the percent egg hatch seven days after treatment for each of the four treatments. The graph shows that Omni Oil® had the lowest percent hatch, followed closely by Ecotrol® at 1.0%. The graph also shows that Ecotrol® at 0.5% and the control had moderate percent hatch and were not significantly different from each other.

Figure 6. Laboratory study, percent egg hatch 7 days after treatment. Error bars are standard error of the mean.

Discussion

Field experiments using botanical oils or products containing botanical oils are scarce (Ibrahim et al., 2001), and therefore the overall goal of this study was to determine the level of efficacy of Ecotrol® (an essential oil blend) on Willamette mite on grapes. The label rate range for Ecotrol® on grapes is 0.375% to 1.5% (concentrated) and 0.1875% to 0.75% (dilute), and the marketing recommendation by the manufacturer is a rate of 0.5%.

Results from the field studies indicate that Ecotrol® at 0.5% is not effective at significantly lowering density of Willamette mite. In the laboratory studies, Ecotrol® at 0.5% was slightly more effective than the control in motile stage mortality, and it was not effective against Willamette mite eggs. However, Ecotrol® at 1.0% was somewhat effective in the field, and very effective against both motile stages and eggs in the laboratory, only slightly less effective than Omni Oil® in killing motile stages, and just as effective as Omni Oil® in killing eggs. In addition, it was found that adding Ecotrol® at 0.5% did not increase the efficacy of Nexter® (pyridaben), currently one of the most common miticides used on Willamette mite.

Spray coverage is always an issue with contact miticide or insecticide applications in the field. This study shows that the lack of efficacy of Ecotrol® at 0.5% in the field was not due to incomplete coverage. As shown by the laboratory study, with 100% coverage, mortality with the 0.5% rate was 32% after 24 hours (the control was 15%) and 73% after 7 days (the control was 63.5%). This can be compared to the 1.0% rate, with 66% mortality after 24 hours and 90.5% mortality after 7 days. Due to the fact that Ecotrol® at 1.0% was effective on adult mites in the laboratory study, it can be expected

that if good coverage is achieved in the field, this treatment would be effective on motile stages in that setting. Sustained spider mite density reduction using miticides also depends largely on efficacy against eggs. Ecotrol® at 0.5% was not effective on mite eggs in the field or in the laboratory, but Ecotrol® at 1.0% was effective on eggs in the laboratory.

The explanation as to why both in the field and laboratory studies, Ecotrol® at 1.0% was more successful than Ecotrol® at 0.5% is unknown. One obvious explanation is that the terpene and terpenoid constituents of the oil blend were not in sufficient concentration at 0.5% to affect the Willamette mite. Perhaps the more studied two-spotted spider mite (for example, El-Zemity et al., 2007) was more susceptible. The mode of action for botanical oils and products containing essential oils (including Ecotrol®) on insects and mites is thought to be an octopamine neuroreceptor blocker (Enan, 2001). This mode of action decreases the binding activity of octopamine receptors, which are responsible for regulation of movement, hemolymph circulation, and overall metabolism. However, one also has to consider that undiluted Ecotrol® is nearly 60% oil. The higher the percent concentration of the spray solution, the greater the effect of a suffocation mode of action.

The laboratory methods used in this research differed from most published laboratory botanical essential oil experiments. In my project, all treatments were sprayed directly on the leaf disks. Attempts were made to treat the leaf disks in the laboratory experiment as if they were conducted out in the field, to simulate a spray application of the treatment. Most other research has been conducted for use in greenhouse settings, for house fumigation, or basic research to analyze efficacy of specific botanical essential oil

constituents, and has used the vapor of the essential oil as the method of treatment (Kim et al., 2004, Aslan et al., 2004). Common methods include use of a small enclosed tube with an essential oil-saturated lid, or dipping or painting the leaf disk in the product and placing the insect/mite on the disk (Miresmailli et al., 2006, Kim et al., 2003, El-Zemity et al., 2007). Other studies used a filter paper diffusion assay which did not allow direct contact with the mite (Choi et al., 2004). An exception was an experiment that measured direct contact toxicity to simulate greenhouse pesticide application using a microsprayer to apply Hexacide® (5% rosemary oil at a 0.75% spray solution, 375 ppm), Ecotrol® (10% rosemary oil at a 0.75% spray solution), Sporan (17.6% rosemary oil at a 0.75% spray solution) and a 100% rosemary oil spray directly on tomato leaves with *T. urticae* (Miresmailli et al., 2006). In that experiment, mortality of adult mites was checked 24h after application. The study found that Hexacide®, Ecotrol®, Sporan® and the pure rosemary oil had an LC₅₀ of 4.01, 5.51, 11.44, and 13.19 ml/liter, respectively. Ecotrol® decreased *T. urticae* populations by 52% after 24 hours. This shows a difference in efficacy according to the percent rosemary oil, which may be due to differences in product formulation. This is also similar to my results, which showed a 32% decrease after 24 hours with a 0.5% formulation. In contrast, another study used a direct contact spray of 25% *Chenopodium ambrosioides* essential oil on adult *T. urticae* and *Panonychus ulmi* and found that at a concentration of 0.5% (1250 ppm) it caused 94.7% and 97.1% mortality, respectively (Chaisson et al., 2004). In my laboratory study, Ecotrol® at 0.5% was equivalent to 600 ppm and Ecotrol® at 1.0% was equivalent to 1200 ppm.

Deleted:

There are several possible reasons why the rosemary/peppermint oil blend used in

these studies was not as efficacious as in other studies. One possibility is the interaction of the terpene or terpenoid components. Some of the individual constituents of the rosemary oil, as well as the complete blend, have been found to be effective against mites. In one study, the full mixture of rosemary oil was found to be more effective on *T. urticae* than various blends of active and inactive individual constituents. El-Zemity et al (2007) found that there was not a large difference between efficacy of essential oils versus individual monoterpenoids on *T. urticae*. That study found that matercary, caraway, garlic, fennel, and rosemary achieved LC₅₀ after 24 hours at 68.39, 141.37, 126.74, 175.45, and 195.09 ppm, respectively. The monoterpenoids borneol, thymol, carveol, and cinnamaldehyde were the most effective with LC₅₀ after 24 hours at 64.12, 111.41, 113.18, and 128.75 ppm, respectively. It should be noted that borneol (a constituent in rosemary oil) was more effective than the rosemary oil.

Phytotoxicity is a concern when using pesticides, and it was thought that the ingredients in Ecotrol® may cause phytotoxicity. On the contrary, we found that Ecotrol® at 0.5% and 1.0% did not cause phytotoxicity, but found that Omni Oil® did even though attempts were made to spray early in the morning at recommended rates (see Photographs 1 and 2). The temperature at the Shandon site may have contributed to the phytotoxicity, as temperatures reached into the 90° F range daily during the study. Waliwitiya et al (2005) found that rosemary oil did not cause phytotoxicity on corn, but thymol, citronellal and eugenol compounds did.

Much work still needs to be conducted on field assessment of botanical oil potential for control of vineyard pests. The use of botanical oils in a pesticide rotation for

resistance management could be applied, if an effective rate is established first (as with Ecotrol® at 1.0%). Different botanical oil compounds should be tested, as well as comparisons to synthetic miticides.

Environmental factors such as temperature and light may increase the rate of product degradation and volatilization of botanical oils (Miresmailli et al., 2006). Some studies show that in an enclosed space (such as a greenhouse or storage bin or silo), the volatilization due to increased temperature may be a beneficial attribute. Kim and Ahn (2001) found that some monoterpene constituents of fennel (E-anethole, estragole and fenchone) were more effective in fumigant studies against the rice weevil with closed cups than open ones. For example, E-anethole reached 100% mortality after 24 hours at 0.42 mg cm⁻² in a closed cup and 0% mortality in open ones. If an enclosed space maintains a higher temperature than open space, and therefore allows the essential oils to volatilize and penetrate the insect or mite via air, then temperature should also be taken into consideration in the field. On the first day (August 16, 2006) of application in my project, the high temperature for the day was 27.8°C (82°F), which is relatively mild for the site. We can speculate that Ecotrol® may have been more effective if sprayed when the ambient temperature was higher, although temperatures within the canopy differ often from the ambient temperature.

If botanical essential oils are to be more widely used in the field, more studies involving spray contact toxicity need to be conducted. Techniques such as filter paper diffusion, slide dipping, and leaf painting determine if any toxicity is present between target pest and botanical essential oil, but are not practical techniques to test field efficacy. Vapor and fumigation techniques might be applicable in greenhouse or storage

setting, and help us to understand how volatile essential oils are.

Appendix A: Photographs

Photograph 1. Phytotoxicity caused by Omni Oil® on chardonnay leaves in the field.

Photograph 2. Phytotoxicity caused by Omni Oil® on chardonnay leaves in the field.

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